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Short Communication

Hypochlorite and Tissue Sterilization

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Summary. Trace amounts of sodium hypochlorite that remain on the surface of seeds (*Lycopersicon esculentum* Mill.) after sterilization interfere with subsequent uptake and incorporation of leucine into protein when the seeds are used in metabolic studies. The hypochlorite can be washed away with 0.01 N HCl but not by washing several times with water.

Sodium hypochlorite (NaOCl) is the most widely used compound for surface sterilizing seeds and other plant tissues before using them in biochemical studies (some recent examples: Ernest and Valdovinos, 1971; Ching, 1973; Jackson and Ingle, 1973; Khan *et al.*, 1973; Nawa and Asahi, 1973). Tissues usually are soaked for several minutes in 0.5–5% NaOCl and then washed several times in water. The effectiveness of this treatment in eliminating seed-borne fungi and bacteria is well recognized, but proof that it does not affect seed metabolism is usually limited to demonstrating that it does not reduce germinability.

We present evidence that only exhaustive washing of the seed with water can remove the NaOCl. The traces of NaOCl which remain after usual washing regimes interfere with uptake and incorporation of leucine into protein.

Samples of 100 tomato (*Lycopersicon esculentum* Mill., cv. Potomac) seeds were soaked at room temperature for 5 min in sterile water (control) or 1% NaOCl (a 5.25-fold dilution of "Clorox", Clorox Comp., Oakland, Calif.[1]). The solution was decanted and the hypochlorite was washed away by (a) washing the seeds 8 times each with 30 ml of sterile water (Table 1); (b) continuous flushing for several hours under running aerated distilled water (Table 2); or (c) soaking for 10 min in 30 ml of 0.01 N HCl followed by washing 8 times with water (Table 3). The seeds were blotted on paper towels and incubated for 2 h at 25° in respiration flasks with 3 ml of 5×10^{-4} M leucine containing 0.6 μCi of [1-^{14}C]leucine (New England Nuclear) plus 60 μg penicillin G and streptomycin sulfate. Decarboxylation of leucine during 2 h incubation was determined by trapping the CO_2 in one drop of 14 N KOH, dissolving the KOH in 1 ml of hyamine hydroxide, and measuring the radioactivity by

1 Mention of trade names is made for identification purposes only and does not imply any endorsement by the United States Government.

Table 1. Removal of NaOCl from the surface of tomato seeds by successive washing in water

Sterilization or corresponding treatment		Metabolism of [^{14}C]leucine during subsequent incubation (dpm $\times$ 10^{-1}/100 seed)				
Treatment (5 min)	No. of washes	CO_2 released	TCA-insol.	TCA-sol.	Total uptake	Incorporation into protein (% of uptake)
H_2O	0	18	258	2310	2568	10.05
1% NaOCl	0	51927	232	665	897	25.86
1% NaOCl	4	19423	369	1384	1753	21.05
1% NaOCl	8	16443	385	1827	2212	17.41

liquid scintillation (Bray, 1960). The seeds were then washed 4 times in cold water and ground in 10% trichloroacetic acid (TCA). Radioactivity in the protein fraction (TCA-insoluble) was determined following standard procedures (see Abdul-Baki and Anderson, 1973). The effect of seed sterilization and removal of NaOCl on germinability was investigated by germinating samples of 100 seeds on paper towels in 10-cm Petri dishes at 25° and 16 h light per day for 5 days. Only normal seedlings with well developed roots were considered as germinated.

The trace amounts of NaOCl in the incubation medium which contained NaOCl-treated tomato seeds and leucine had two distinct effects on the uptake and incorporation of the amino acid into protein. The first effect was the decarboxylation by NaOCl of leucine into CO_2 (Tables 1–3) and other products which we identified as isovaleraldehyde and isovaleric acid. This reaction took place in the incubation medium and was independent of the presence of seeds (Table 3). Thus, the produced CO_2 was non-metabolic. Decarboxylation of leucine reduced its concentration in the incubation medium and resulted in a reduced content in the free amino acid in the seed (TCA-soluble fractions in Tables 1–3). The second effect was a stimulation of leucine incorporation into protein in seeds disinfected with NaOCl. This effect was detectable even when uptake was severely reduced, and the difference was particularly obvious when compared with treatments where NaOCl was completely removed either by exhaustive washing with water or by HCl (TCA-insoluble fractions of Tables 1, 2).

The overall effect of disinfecting the seeds with NaOCl on uptake and incorporation of leucine into protein depends on how well the hypochlorite is washed away before the seeds are incubated in leucine. The data clearly show that even after washing the seeds up to 8 times with water there was still enough NaOCl on the seeds to alter uptake of the amino acid and its incorporation into protein (Table 1). Continuous washing for several hours under running water, a treatment injurious to seeds of many

Table 2. Removal of NaOCl from surface of tomato seeds by continuous washing with aerated water for different time periods

Sterilization or corresponding treatment		Metabolism of [^{14}C]leucine during subsequent incubation (dpm $\times 10^{-1}$/100 seed)				
NaOCl (5 min)	Washing time (min)	$^{14}CO_2$ released	TCA-insol.	TCA-sol.	Total uptake	Incorporated into protein (% of uptake)
−	0	32	276	2354	2630	10.5
+		56632	460	1144	1604	28.7
−	20	32	266	2792	3058	8.7
+		8602	504	2166	2670	18.9
−	90	34	292	3204	3496	8.4
+		1156	540	2804	3344	16.2
−	270	30	386	4690	5076	7.6
+		188	566	3824	4390	12.9

Table 3. Removal of NaOCl from surface of seeds by washing in water or HCl

Sterilization or corresponding treatment	Metabolism of [^{14}C]leucine during subsequent incubation (dpm/200 seeds)					
	CO_2	TCA-insol.	TCA-sol.	Total uptake	Incorporated into protein (% of uptake)	Germination (%)
$H_2O \rightarrow 8$ washes	310	906	3730	4636	19.6	92
1% NaOCl → 8 washes	97530	1766	3430	5196	34.0	94
1% NaOCl → HCl → 8 washes	930	1270	4430	5700	22.7	95
−seed, $+H_2O$	80	—	—	—	—	
−seed, +NaOCl	91337	—	—	—	—	

species, did not completely eliminate the effects of NaOCl either, as evident from increased CO_2 production and lowered uptake of leucine (Table 2). On the other hand, soaking of NaOCl-treated seeds for 10 min in 0.01 N HCl, followed by several washings with water, reduced decarboxylation of leucine without affecting germinability (Table 3).

We conclude that NaOCl, when used, must be removed from the tissues completely with an acid treatment before the tissues are used in metabolic studies, such as those that call for the use of labeled amino acids.

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